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NEW FACTS AND VIEWS CONCERNING THE OCCURRENCE OF A SEXUAL PROCESS IN THE MYXOSPORIDIAN LIFE CYCLE.¹

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THE classic observations of Balbiani, Bütschli and Thélohan on the myxosporidian development do not include the occurrence of a sexual process which comprehends the forming of a syncaryon in the life history of this protozoan group. Doflein (1898 and 1901) suggested *two* places in the life cycle of the myxosporidian where a caryogamy might probably take place. In the next period of investigations on myxosporidia the occurrence of this sexual process was stated by various authors, but they differ widely in the conception of the place in the life cycle in which the copulation occurs. Mercier, Awerinzew,

¹ For a clear understanding of the question under discussion, it is necessary to have a uniform nomenclature and to discard all terms which are only of historical value. Noyaux du sporoplasma, noyaux du germe, noyaux sporoplasmiques, germnuclei, Amöboidkeimkerne were to be discarded and gametonuclei, noyaux des gamètes, Gametenkerne were to be used. Instead of sporoplasma, sporoplasm or amöboidkeim, only such expressions as gametes, gametes and Gameten are admissible. Identical and adequate terms are capsulogenous cell, cellule capsulogène, Polkapselzelle. Valve cells, cellules valvaires and Schalenzelle should be used for those cells which form the membrane of each single spore; cellule d'enveloppe, envelope cells, and Hüllzellen should be used for those cells which form the membrane of the pansporoblast. In cases where only the nuclei of these cells are present, the terms noyaux d'enveloppe, envelope nuclei, Hüllenzellenkerne should be substituted. If the cellular origin of the pansporoblast membrane is not ascertained, envelope, membrane d'enveloppe or Hülle may be used. The terms Restkern, residual nucleus are misleading. Somatic residual nuclei or somatische Restkerne should be used, if the definition on p. 679 holds true. Here a new name would be of great value to eliminate the wrong analogies created by Doflein (1898, p. 309). The term "reduction nuclei" is only justified if the numerical reduction of chromosomes has been ascertained.

Auerbach and Parisi try to show that a real syncaryon formation takes place at the *onset* of spore formation. Other authors, Keysselitz, Schroeder and Auerbach, believe that only a plasmogamy can be pointed out at the beginning of spore formation, and that the union of the nuclei is effected either in the fully developed spore or in the young animal leaving the spore. The difference between these last two conceptions is theoretically without significance because the main part of copulation—the union of the nuclei—takes place *at the onset of the new life cycle of the myxosporidian*. Therefore it was of the utmost importance for decisive proof of this fact to find the copulation of the two gametonuclei inside the fully developed spore or in the young myxosporidian. Schroeder, 1909, observed the copulation of the two gametonuclei in the spore; Auerbach, 1907 and 1910, found young animals of *Myxidium bergense* with one nucleus. I was able to demonstrate young *Chloromyxum leydigi* which were experimentally produced by placing the two-nucleated spores on gall plates (Erdmann, 1911). Here, after a treatment with intestinal secretions of the host the young animals leave the spore. They are at first binucleate, later uninucleate. In my recent work, finished in 1913, which did not appear until 1917 in consequence of the war, I figured these young animals after fixation and staining. Also, Davis, 1915, though with some reserve, presents young *Sphaerospora dimorpha* which have left the spore and show the fusion of their two nuclei. Later the separation of the syncaryon into its vegetative and generative components takes place. Georgevitch, 1914, presents the development of the young animal in *Henneguya gigantea* in—as it seems—changed peculiar conditions. The spore is still inside of the cyst of the big “tumor-forming” tissue-parasite. The binucleate form becomes uninuclear and then the usual vegetative multiplication of the nuclei begins, which leads up to a *renewed* spore formation *inside* of the tumor cyst. In *Chloromyxum leydigi*, a gall-bladder parasite, no such

complicated process takes place. As the young uninucleated forms develop we see an animal with three nuclei, all of the same size. Later multiplication of these vegetative nuclei and the formation of big syncytial masses occur. The plasmatic bodies of these vegetative animals contain two kinds of round corpuscles, "Reservekörper" and "Farbträger." In my publication in the *Archiv für Protistenkunde*, 1917, I give proof that the "Reservekörper" consists mostly of glycogen and I point out that the glycogenous contents are used up during spore formation. The vegetative animal can multiply either by division or by forming small vegetative gemmules (Erdmann, 1911). The fact that inside the animal vegetative propagative bodies can arise, was verified by Davis, 1915, pp. 354-355, in *Sphaerospora dimorpha*.

Before the onset of spore formation a differentiation in the syncytial masses of *Chloromyxum leydigi* begins. We can distinguish parts in which the nuclei multiply and other parts where only the vegetative nuclei are seen widely scattered in the protoplasm. I called the first-mentioned areas "islands" (Erdmann, 1911) because in the living animal they rise above the surface of the vegetative plasmatic body. They are distinguished by their pale color and in stained preparations by their large number of small nuclei. At first all the nuclei in these islands are of the *same* size. Two nuclei with small cytoplasmic bodies approach each other and each cell divides up into a small and a big cell. The two small cells draw out in length and surround the two big ones, in this manner separating them from the other cells in the island. This quadruple group, two big cells and two small ones, is the starting point for the formation of the whole spore. The two big cells are gametocytes. These two gametocytes divide and form two gametes and two other cells which after a further division give rise to four cells—these four cells are the four capsulogenous cells. The whole spore contains, therefore, eight cells—four capsulogenous cells, two gametes and two cells which form the spore membrane. (Fig. 1.)

I mentioned before that the glycogen which was found in the vegetative body is used up in spore formation. The membrane of the spore, the polar threads and the darkly staining structureless lumps, which have been seen by all authors inside the sporoblast, consist of glycogen and stain as well by chromatin as by specific glycogen stains. These lumps have been considered as "reduction nuclei" by various authors. They have also been called "Restkerne" or "residual nuclei." It may be emphasized for later discussion that they are glycogenous and not chromatic in *Chloromyxum leydigi*.

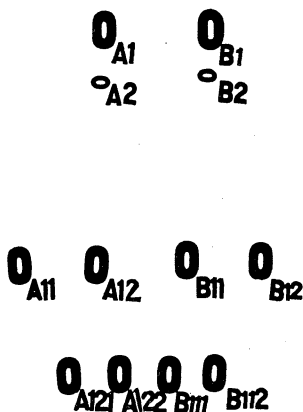


FIG. 1. *Chloromyxum leydigi*: A_2 and B_2 are the spore membrane forming cells; A_{11} and B_{12} are the gametes. A_{121} A_{122} ; B_{111} B_{112} are the capsulogenous cells.

Our knowledge of the sexual process in the myxosporidian life cycle since the investigations of Keysselitz, Schroeder, and others has been completed by recent authors: Georgevitch, 1914, Davis, 1915 and Mavor, 1916. The best proof that *no* reduction takes place in the spore is given by Georgevitch (1914) and Davis (1915), who have been able to study the number of chromosomes in their specimens before and after spore formation. Both authors agree that the number of chromosomes is not changed before and after this phenomenon. Davis figures six chromosomes in *Sphaerospora dimorpha* and Georgevitch, investigating *Henneguya gigantea*, finds eight. These two facts: *first, that the number of chromosomes is not changed in spore formation and, second, that the so-called "reduction nuclei" inside the spore are really glycogenous bodies which are used up in forming the membrane of the spore, polar threads, and spore membrane of Chloromyxum leydigi,* positively prove that the real reduction must occur at a different place in the life cycle of *Sphaerospora*, *Chloro-*

myxum, and *Henneguya*. The reduction consisting in the transformation of a diploid nucleus into a haploid or of a tetraploid one into a diploid can only occur at the beginning of the new life cycle *after* or *before* the union of the gametonuclei.

The further facts which Davis, 1915, presents in *Sphaerospora dimorpha* also tend to show that reduction could only take place at the above outlined place. The gametonuclei of this myxosporidian fuse together after it leaves the spore and form by one subsequent division *two* nuclei. One of these nuclei gives rise to *all* the cells of the later sporogenous body. The other of these two nuclei, distinguished by its size and structure, is the vegetative nucleus of the animal, the somatic "Restkern." *No other nuclei should be called "Restkerne" except when they represent the nucleus or nuclei of the vegetative myxosporidian body, which does not play a part in spore formation.* Such "Restkern" or "Rest-

TABLE I

Author	Species	Occurrence of Somatic Residual Nuclei in Sensu-stricto, See Definition, p. 679	Occurrence of Hüllzellen Envelope Cells, Pan-sporoblast-membrane Forming Cells or Only Their Nuclei	Number of Valve Cells. They Are Division Products of Gametocytes
1. Awerinzew.	<i>Myxidium</i> sp.	One	—	2 for each spore
2. Auerbach...	<i>Myxidium bergense</i>	None (p. 26)	—	"
3. Davis.....	<i>Sphaerospora dimorpha</i>	One, seldom two	—	"
4. Awerinzew.	<i>Ceratomyxa drepanop-sette</i>	Two	—	"
5. Mavor.....	<i>Ceratomyxa acadiensis</i>	Two	—	"
6. Erdmann...	<i>Chloromyxum leydigi</i>	Many	—	"
7. Davis.....	<i>Sphaerospora dimorpha</i> , polysporous form	Many	—	"
8. Auerbach...	<i>Myxidium bergense</i> , polysporous form	None	—	"
		(It may be that Auerbach has observed in his mono-, di- and polysporous forms only the propagative parts of the complete animal.)		
9. Parisi.....	<i>Sphaerospora caudata</i>	No facts mentioned	Two	"

kerne," the origin and fate of which agree with this definition, have been described by Awerinzew in *Ceratomyxa drepanopsetta*, and in *Myxidium* sp., by Davis in *Sphaerospora caudata*, and by Mavor in *Ceratomyxa acadiensis*.

In the disporous form, *Sphaerospora dimorpha*, two spores are found in the whole animal and the sporogenous body finally contains twelve cells—half of this number forms one spore (Fig. 2). These twelve cells are all

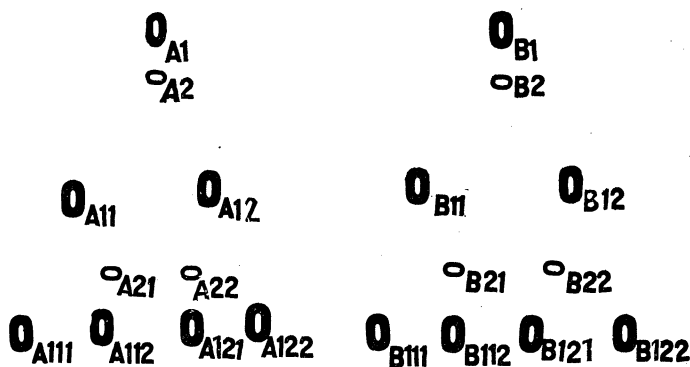


FIG. 2. *Sphaerospora dimorpha* (disporous form): A_{21} A_{22} and B_{21} B_{22} are the membrane-forming cells for each spore. A_{111} A_{112} ; B_{111} B_{112} are the two gametes in each spore. A_{112} A_{121} ; B_{112} B_{121} are the two capsulogenous cells in each spore.

division products of the *one* nucleus, the *sister* nucleus of which is the somatic "Restkern." The cells which form the spore membrane of each spore, lying independently in the vegetative body, arise by a very *late* division. Chromatic lumps which could be considered as "reduction nuclei" are lacking. It is easy to imagine why they are absent. *Sphaerospora dimorpha* lives in the urinary bladder and has therefore a metabolism which may not afford opportunity for an abundant glycogen formation. Also, as mentioned before, no reduction of the chromosomes takes place. These facts could be easily ascertained in consequence of the large size of the cells and chromosomes. Awerinzew's description of spore formation in *Ceratomyxa drepanopsetta* and Mavor's of *Ceratomyxa acadiensis* have many features in common, but Awerin-

zew's presentation differs from that of the other author in one important point. The cells *A* and *B* in Fig. 2 are said to be in *Ceratomyxa drepanopsettae* the products of a fusion of two cells. The reader has but to change the lettering of *A* in *AC* and of *B* in *BC* to see that the two copulae undergo changes identical with the two single cells in *Sphaerospora dimorpha*. Except this one point of difference—the beginning of spore formation—we note that the late division of the membrane-forming cell, the identical number of cells in each spore and the independence of each spore in the myxosporidian body characterize both species of *Ceratomyxa* (Awerinzew and Mavor) and *Sphaerospora* (Davis). It appears highly improbable that in two different species of *Ceratomyxidae* the basis of spore formation should be a copula (Awerinzew) or an univalent nucleus (Mavor).

Summarizing the known description of spore formation of those myxosporidia, in which *each spore is formed independently of the other in the somatic body*, and where no pansporoblast exists, we can demonstrate the following uniform features in all investigated forms.

1. Six cells or nuclei are used for the formation of each spore, when two polar capsules are present; eight, when four polar capsules are present.

2. The cells which form the spore membrane have a similar origin and are distinguished by the independence in which these cells develop as compared with the other constituents of the spore. Their mother cell is lying in a resting stage till the division of the gametocyte is finished, as described by Davis for *Sphaerospora dimorpha*, and by Awerinzew for *Ceratomyxa drepanopsettae*.

In *Myxidium* sp., where, according to the investigations of Awerinzew, either one, two or three spores are lying independently in the myxosporidian body, there is a very late division of the cell, the divisional products of which form the spore membrane as recorded by this author (Fig. 3). Here the one gametocyte divides into two cells, one of which by a late division gives rise to the two-

spore membrane-forming cells, the other forms the two capsulogenous cells and two gametes. We intentionally

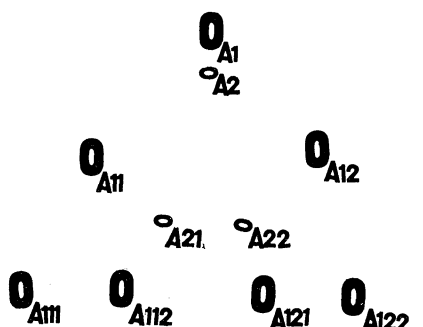


FIG. 3. *Myxidium* sp.: A_{21} and A_{22} are the spore membrane-forming cells (valve cells). A_{111} A_{122} are the gametes. A_{112} and A_{121} are the capsulogenous cells.

avoid, in Fig. 3, calling the two chromatin lumps, which the author himself (p. 202) has called "überflüssiges Chromatin" (degenerierende Kerne), nuclei and do not add them as such in the diagram as Auerbach, 1912, p. 28, did when discussing Awerinzew's investigations. We consider

these chromatin lumps only as formations probably of glycogenous nature and as being used during membrane formation.

In Fig. 4 Auerbach's conception (Type I) of how the spore formation is effected in *Myxidium bergense* is represented. Auerbach believes, as stated before, that either a plasmogamy (Type I) or a real copulation (Type II) may be at the basis of spore formation. We will not discuss, for the present, how the bigger and smaller cells which are seen at the beginning of spore formation, arise. The latter divides once and the two divisional products form the spore-membrane-forming cells. The other cell divides twice to give rise to two gametes and two capsulogenous cells.

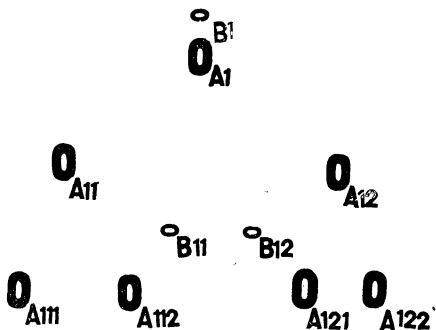


FIG. 4. *Myxidium bergense* (Auerbach Type I); B_{11} and B_{12} are the spore membrane-forming cells (valve cells). A_{111} and A_{122} are the two gametes. A_{112} and A_{121} are the capsulogenous cells.

The author does not especially mention the order in which these cells divide,

but nothing in his report contradicts the supposition that in *Myxidium* sp. and in *Myxidium bergense* there is a close analogy with Davis's and Awerinzew's observations. He also observes the elimination of chromatin and "eventuell Bildung von Restkernen" (p. 24). These are not incorporated in Fig. 4 for the same reasons I pointed out for *Myxidium* sp.

My observations in *Chloromyxum leydigi* show, furthermore, that the cells which form the valves of the spore do not play a part in the development of the final contents of the spore, but are here in this form (comp. Fig. 1) the products of the first division of each gametocyte. In the case of *Chloromyxum leydigi* two gametocytes form by one division the two spore-membrane-forming cells; in the three other species, *Myxidium* sp. (probably *Myxidium bergense*), *Ceratomyxa drepanopsettae* and *Sphaerospora dimorpha*, one gametocyte forms the one cell, the division products of which are transformed into the valves of the spore. But in all four species these cells have the sole purpose of forming the spore membrane.

After surveying the Figs. 1, 2 and 3, and having compared them, I have no doubt that the origin of the spore-membrane-forming cells is identical in the so-called monosporous and disporous forms. Advancing one step farther and taking into consideration those forms in which two gametocytes form the cells inside each spore (Fig. 1) we notice that in dealing with the origin and the position in the development of the spore, we have to add nothing. The spore-membrane-forming cells are distinguished by their early segregation from the gametocytes and their non-entering into the series of those cells which are included in the spore. The only difference is that these cells do not divide further; if they did, we could easily construct the disporous type of *Sphaerospora dimorpha* (Fig. 2). The same holds true for those species which form two spores in one pansporoblast (Fig. 4) and where two gametocytes are observed at the basis of spore formation (Keysselitz, Schroeder).

If we conceive these two cells in question (A_2 and B_2) which form in *Myxobolus pfeifferi* (Keysseltz) the pansporoblast membrane to divide once more and the last division inside the spore to be suppressed, we could have the type of *Sphaerospora dimorpha*.

According to Keysseltz, in *Myxobolus pfeifferi* each of the two gametocytes together with the small cell ap-

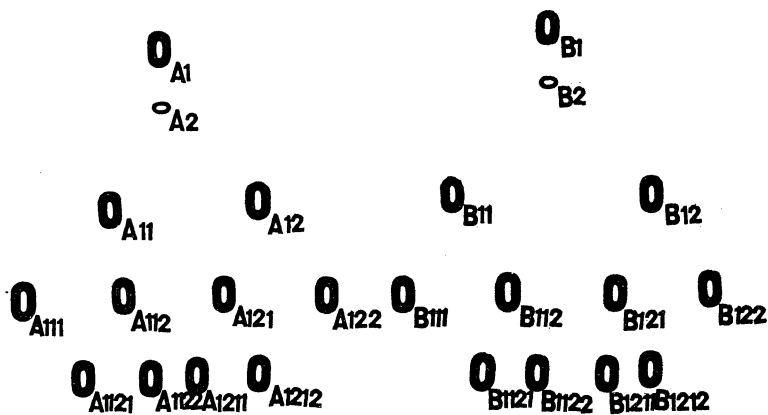


FIG. 5. General plan for polysporous disporoblastic forms. (Type Keysseltz.) A_2 and B_2 are the envelope-forming cells (pansporoblast-forming cells). A_{111} and A_{122} ; B_{111} and B_{122} are the two gametes in each spore. The products of the fourth division form valve cells A_{1121} , A_{1211} , B_{1121} , B_{1211} , and capsulogenous cells A_{1122} , A_{1212} , B_{1122} , B_{1212} .

proach each other and each divides up until six cells have arisen. Thus we have in all cases 14 cells, two of which have a different divisional capacity, for they stop dividing and the big cells form all the other cells which at the end compose the two spores. Their later fate is indicated in Fig. 5 and, though this does not concern us in this discussion, we should like to emphasize the fact that two gametes are always present in a certain stage of spore development. We are convinced that the cells A_2 and B_2 represent genetically in the pansporoblastic forms that cell formation which in all monosporous, disporous and polysporous species gives rise to the spore membrane itself (Figs. 1 to 4). These cells or their nuclei were observed by Keysseltz, Parisi, Lo Giudice, Auerbach (*Henneguya psorospermica*), Georgevitch, and with cer-

tain restrictions by Mercier and Schroeder. This cell couple (A_2 and B_2) should be called envelope cells or envelope cells nuclei when they do not fulfill their destiny (Schroeder, probably Mercier). Even in those cases in which a pansporoblast membrane had not been discovered it might have been either overlooked or have been in evidence at the beginning of pansporoblast formation before the valves of each single spore had been developed. Later these take up the function of the Hüllzellen which make their retrogressive development plausible. These Hüllzellenkerne are neither Restkerne, nor reduction nuclei, nor somative residual nuclei. The term for residual nuclei of somatic nature (see definition, p. 679) has already been disposed of in *monosporous* and *disporous* forms and must be used in the same way in polysporous forms. In all forms which are polysporous and have many singly developing spores, the whole vegetative body which is not used up in spore formation has somatic "restkerne" or residual nuclei. As I pointed out in *Chloromyxum leydigi* the vegetative animal may die after spore formation together with the "restkerne." In this form the vegetative animal may prolong its life by forming internal buds, if it has reached a considerable size before and during spore formation. In all polysporous forms with pansporoblast, *i. e.*, the disporoblastic forms, we have to be very careful when applying the name of somatic residual nuclei. In those species which are not tissue parasites and sometimes have cystlike formations which are surrounded by gelatinous envelopes, we may find somatic residual nuclei, because it seems improbable that the whole vegetative body is used up for spore formation. I believe this to be the case in *Sphaeromyxum sabraezesi* and *Sphaerospora caudata*. Where no residual vegetative nuclei were observed, the investigators may not have studied the whole animal, but only the propagative parts of it which have left the vegetative body (Parisi, Fig. 3). Keeping this point in mind, later investigators may discover somatic residual nuclei in de-

generating stages analogous to those found in the monosporous, disporous and the non-disporoblastic polysporous forms, among the debris of the dying animals inside the gall and urinary bladder, as I have shown in *Chloromyxum leydigi*. In the so-called "tumor-forming" disporoblastic polysporous forms, no *facts* are known which show that somatic residual nuclei have been observed. The beginnings of cyst formation, however, have never been studied, and it is only at this stage that somatic residual nuclei may be seen, and not after the cyst is crowded with sporoblasts and spores. But even in the fully developed cysts there may be degenerating somatic residual nuclei which have escaped observation. The facts which Weissenberg found in *Glugea anomala* and *hertwigi*—two Microsporidia—seem to support my suggestion. But the Hüllzellen or Hüllzellenkerne of the myxosporidia are never identical with somatic nuclei. Their undisputed place in the development of the myxosporidia will soon be clear.

Before we proceed further in discussing the formation of the sporoblast membrane in those myxosporidian species in which the spores are formed in *pairs* inside one sporoblast, it may be recalled that several facts have been ascertained concerning the fully discussed spore-membrane formation in monosporous or disporous myxosporidian species: (1) The copulation of two gametes occurs during or after the two myxosporidia leave the spore. (2) No reduction takes place from the beginning of spore formation until the end, because the number of chromosomes remains the same (Davis and Georgevitch). (3) The darkly staining masses of "restkerne," "residual nuclei," "reduction nuclei" have been shown to be glycogenous and to be necessary for spore-membrane formation. (4) Membrane-forming cells or nuclei are set apart by different division intervals from the other cells of the sporoblast.

Now from the above given summary of the latest facts in monosporous or disporous forms, it is clear that they

are strictly *opposed* to all the views which maintain that a copulation or so-called syncaryon formation precedes spore formation. But they are all in accord with the investigations of all authors who have shown that there is no syncaryon formation, but merely a plasmogamy of two cells, without any copulation, at the onset of spore formation.

When I wrote the second part of my investigations on *Chloromyxum leydigi*, 1913, I pointed out that the facts which were presented by Auerbach, Mercier and Parisi, as proofs of the occurrence of a syncaryon formation just before the onset of spore formation, are not quite convincing. Their figures can easily be arranged in such a manner that the supposed syncaryon formation represents the division of gametocytes into a smaller cell, which in most all other known cases forms the membrane of the pansporoblast. (Compare Erdmann, 1917.) It is not necessary to repeat here the attempted revision and rearrangement of the figures of these authors. This same holds true for the syncaryon formation in *Myxidium bergense*, Type II (Auerbach) and *Ceratomyxa drepanopsettae* (Awerinzew). We will take it for granted that our views are correct as long as no new *facts* ascertained on smears—not sections—compel us to change our opinion.

As mentioned above, all authors who have shown that no syncaryon formation occurs, but that a plasmogamy of two cells without any nuclear fusion occurs at the onset of spore formation, can agree with us that the sexual process is going on at the beginning of the new life cycle. Auerbach and Parisi do not convince us that the figures which represent the so-called caryogamy can not be considered as the dividing of the gametocyte in the two cells. In accordance with the facts and interpretations, Keyselitz, Schroeder, Lo Giudice, Erdmann, and Georgevitch uphold the view that *no* merging of two cells or two cell pairs takes place to form the couples of cells which are later considered as a quadruple group of the growing spore. By comparing the series of figures of all those drawings

which are supposed to prove the merging of two cells, they can, as said before, be interpreted as the division of one cell into two. The larger of these cells, wrongly called macrogametocyte according to the cell fusion theory, has divided and formed the cell wrongly called microgametocyte.

This "microgametocyte" and its division products after one division, or these "microgametocytes" in cases where two gametocytes are observed at the onset of spore formation (Keysselitz, Schroeder, and Erdmann) now form, according to all known investigations, the pansporoblast membrane. Just as we could point out in disporous forms the uniformity of the origin of the spore-membrane forming cells (Figs. 2, 3 and 4) so we can do the same for the pansporoblast membrane and its nuclei in the following forms: *Myxobolus pfeifferi* (Keysselitz), *Myxobolus ellipsoides* (Lo Giudice), *Sphæromyxa sabrazesi* (Schroeder), *Sphærospora caudata* (Parisi), *Henneguya gigantea* (Georgevitch), and *Henneguya psorospermica* (Auerbach); all following Fig. 5, provided we do not take into consideration the origin of the cells A , A_2 and B and B_2 . Keysselitz and Schroeder's views, except one contradictory point, are exactly represented by Fig. 5, but there are differences mentioned by the other authors. Still we make the generalization that there is one and the same plan of spore formation in the pansporoblastic myxosporidia though we know that facts are reported which do not fit in with our view. *We hold the opinion that it is permissible to rearrange the observed facts, because all interpretations have been gained by piecing together and arranging facts according to the theoretical viewpoint of the authors.* No continued observation of spore formation in the living animal has been possible. Also we are allowed to add facts ascertained in other species if the authors have only considered sections and not smears. Sections are misleading because the whole quadruple group can not always be seen on the same section and the origin of the small cell from

the big cell can not be traced without doubt. Therefore, most investigators have lately used smears to get a fuller and more correct view of the origin of the different cells from each other. It is astonishing how scanty the details appear when one considers the formation of the quadruple group in Auerbach's, Lo Giudice's, Parisi's and Georgevitch's presentations. Mercier's Figs. 19-27, Plate 1; Auerbach's Figs. 8a-15, Plate 2; Lo Giudice's Figs. 29-34, Plate 1; Parisi's Figs. 13-18, Plate 16; and Georgevitch's Figs. 32-35, Plate 1, do not show each single step of this important process. Connecting stages are missing. Therefore, one is allowed to interpret differently *their* presented facts, as I have done in Fig. 5. In Table II

TABLE II

I	II
<i>Myxobolus pfeifferi</i> Mercier.	<i>Sphaeromyxum sabrazesi</i> Schroeder.
Cell <i>A</i> (copula) forms all other 12 cells of the pansporoblast and the two "Hüllzellenkerne."	<i>A</i> ₂ <i>B</i> ₂ <i>A</i> ₁ <i>B</i> ₁
	All cells divide up to form the 12 pansporoblastic cells and the two "Hüllzellenkerne."
<i>Myxobolus ellipsoides</i> Lo Giudice.	<i>Henneguya gigantea</i> Georgevitch. <i>Henneguya psorospermica</i> Auerbach (Type I).
	<i>A</i> ₂ <i>A</i> ₂₂ <i>A</i> ₁ <i>A</i> ₁₂
	Cells <i>A</i> ₂ and <i>A</i> ₂₂ do not form any of the other 12 cells of the pansporoblast.

we can study the different opinions from the authors' point of view. Lo Giudice, Georgevitch, Auerbach, Keyselitz, and Schroeder are alike in interpreting that the two cells which develop into the pansporoblastic membrane or nuclei are separated very early from the other cells. They never intermingle with those cells inside the spore-membrane (except according to Schroeder). They can not, therefore, be microgametocytes and in consequence they have nothing whatsoever to do with a sexual phenomenon. This adds strong support to our view that

the sexual process is at the beginning of the new life cycle.

I do not wish to veil the great discrepancy between the conception of Mercier (Fig. 6) and the other authors

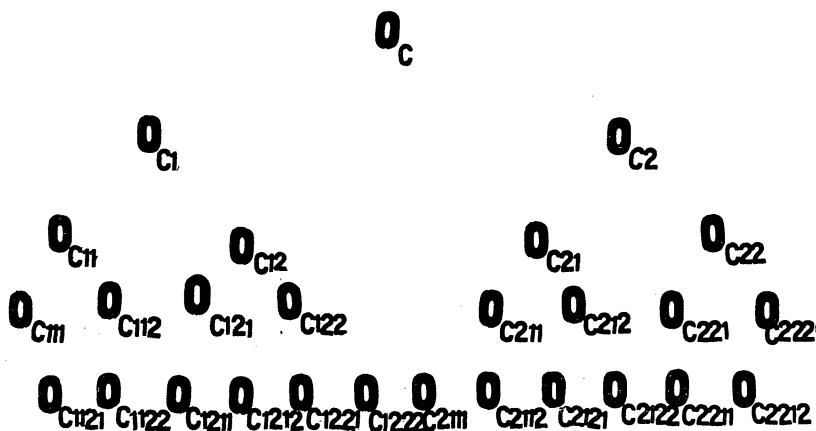


FIG. 6. *Myxobolus pfeifferi*, Mercier: C_{111} and C_{222} are the celles d'enveloppe of this author, which represent the false "Restkerne" or "reduction nuclei" of other authors. The products of the fourth division form, for each spore, the gametes, the valve cells and the capsulogenous cells.

mentioned. All cells are products of a copula, and there is no setting apart of the pansporoblast-membrane-forming cells or nuclei, though later they appear at the accustomed places between the two spores (Plate I, Figs. 31, 32). I shall not risk an interpretation, but think a new investigation on this same subject might be very promising and result in the desired uniformity. I think it highly probable that in all pansporoblastic forms the spore development follows the Keysseltz-Schroeder interpretation: that two gametocytes form the basis of the spore formation. But even if one believes that the quadruple group is not formed by two but by one cell pair, the principal point is not changed. It is indifferent for the theoretical interpretation whether a segregation of the second cell pair from the first takes place, and both then form the quadruple group, or whether two cell pairs approach each other and form the quadruple group. The pædogamy is merely a closer one in the first case.

To summarize: In the observed myxosporidian species

with pansporoblast (exception, Mercier, *Myxobolus pfeifferi*), the first division products of the gametocyte or gametocytes form the pansporoblastic membrane or, if degenerated, its nuclei. This division is a heteropole division and forms highly chromatic small cells or nuclei which never intermingle with the cells inside the pansporoblastic membrane (exception, Schroeder).

On the basis of these facts, we need only state that the heteropole division has no connection whatsoever with a reduction division, as Keysselitz tentatively suggested. This conspicuous division produces the Hüllzellen or Hüllzellenkerne, and it may not be impossible that in the case of *Sphaeromyxa sabrazesi* these small chromatin cells do not intermingle with the others and divide up, though the author mentions it on page 366. They originate, according to Schroeder's second interpretation, in the same manner as most authors describe, but divide together with the big cells until twelve cells are present in the pansporoblast; they then take their accustomed place inside the sporoblast membrane and are easily recognizable. This apparent exception merits further investigation.

We maintain our conclusion that the Hüllzellenkerne or the Hüllzellen are identical with the spore membrane forming cells of the non-disporoblastic polysporous forms. They have the following features in common: they are the first division products of the gametocytes; they do not intermingle with the other cells inside the spore; they form the envelope, in one case of the spore, in the other of the pansporoblast. They are neither somatic residual nuclei nor Restkerne nor reduction nuclei. They are cells which have a tendency to degenerate in some disporoblastic forms when their functions are taken up at an early period by the valve cells or spore-membrane-forming cells.

It remains, as the last part of our discussion, to deal fully with the significance of those darkly staining masses which have been described as "Reductionskerne" or "Restkerne" "*inside* the sporoblast." In the following table we give a short survey of the known facts.

A survey of Table III brings out clearly certain facts. When darkly staining masses are observed inside the

TABLE III

Author	Species	Occurrence of Chromatic Bodies Inside the Spore Interpreted as Reduction
1. Awerinzew	... <i>Myxidium</i> spec.	Seldom distinct small nuclei, generally "Zwei ziemlich grosse Chromatinkügelchen" (p. 201).
2. Auerbach	... <i>Myxidium bergense</i>	Diffusion of chromatin or formation of two "restkernartigen Gebilden" (p. 20).
3. Davis	... <i>Sphaerospora dimorpha</i>	Formation of round chromatic bodies.
4. Awerinzew	... <i>Ceratomyxa drepanopsettæ</i>	Infiltration of chromatic small bodies into the cytoplasm before the spore membrane includes the gametocytes after the supposed copulation. Note here the later formation of spore membrane.
5. Mavor	... <i>Ceratomyxa acadiensis</i>	Formation of round chromatic bodies at the onset of spore formation (Fig. 10) which are later resolved.
6. Erdmann	... <i>Chloromyxum leydigi</i>	Formation of several large, deeply staining bodies which disappear after the spore membrane is formed.
7. Parisi	... <i>Sphaerospora caudata</i>	Formation of small, deeply staining bodies before the spore membrane includes the gametocytes after the supposed copulation (Fig. 15).
8. Davis	... <i>Sphaerospora dimorpha</i> , polysporous form	No diffused infiltration of chromatin observed, also no formation of round chromatic bodies.
9. Auerbach	... <i>Myxidium bergense</i> , polysporous form	Formation of two "restkernartigen Gebilden" or diffusion of chromatin.

10. Keysselitz ... *Myxobolus pfeifferi* One to four round chromatic, deeply staining bodies, disappearing after the valves of the spores are formed (p. 261).
11. Mercier *Myxobolus pfeifferi* Diffusion of small chromatic bodies into the cytoplasm (Figs. 33, 34) after the sporoblast membrane is formed, also the same fact stated after the supposed copulation (Figs. 22, 23).
12. Lo Guidice ... *Myxobolus ellipsoides* Several round chromatic, deeply staining bodies, which are not observed after the spore membrane is formed (Figs. 41, 42).
13. Schroeder *Sphaeromyxum sabrazesi* ... Chromatic, deeply staining bodies which are not observable when spore membrane is formed. (Comp. Figs. 30, 32 with Figs. 33, 34.)
14. Auerbach *Henneguya psorospermica* .. To judge after Figs. 6 to 18, extrusion of a chromatic body in cytoplasm. (Note, only sections to judge from.)
15. Georgevitch .. *Henneguya gigantea* Four deeply staining chromatic bodies called by the author degenerated nuclei.

spore, they disappear after the spore membrane is formed. It is proved that in *Chloromyxum leydigi* they are of glycogenous nature as well as the spore membrane itself and the polar threads. In some cases their number is irregular. These chromatic lumps may be products of nuclear division, but the true chromosomes have not been found. Those authors (Mercier, Awerinzew, Parisi) who believe they have shown a syncaryon forming, have also observed an extrusion of chromatin immediately after the union of the supposed micro- and macro-gametocyte. In Mercier's case a second diffusion of round bodies is shown *inside* the spore which corresponds with the facts observed in other species. *Ceratomyxa drepanopsettae*

(Awerinzew) has only an extrusion of chromatin before the syncaryon formation, while in *Myxobolus ellipsoides* (Parisi) it occurs immediately after this phenomenon. These exceptions in the series, *i. e.*, that inside the spore no chromatin diffusion is observed, may be due in the case of Parisi to a limited number of studied forms and in the case of Awerinzew to the fact that the spore membrane in *Ceratomyxa* is formed very late. Yet these exceptions do not prevent the final statement that the darkly staining chromatic masses in the spore are not reduction nuclei, or restkernartige Gebilde, but play an important part in the development of the spore membrane.

The whole trend of our critical review leads up to the following conclusions:

1. Reduction in myxosporidia has thus far not been discovered.

2. The so-called reduction nuclei inside the spore are chromatic or glycogenous masses, which serve the spore-membrane formation.

3. The so-called residual nuclei of the disporoblastic forms can not be considered as identical with the somatic residual nuclei of the mono-, di- or poly-sporous non-disporoblastic forms. They are the functionless nuclei of the envelope cells of the disporoblastic forms.

4. The envelope cells can by their origin only be compared with those cells in the mono-, di- or polysporous nondisporoblastic forms which later give rise to the valve cells.

5. The somatic residual nuclei are well-defined in mono-, di- or poly-sporous nondisporoblastic myxosporidia. Their analogy has not thus far been discovered in disporoblastic polysporous forms.

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* In consequence of the war I did not see my own reprint printed in the *Archiv für Protistenkunde* so it is impossible to add volume and page reference.